Disruption of Reproductive Activity of *Coptotermes formosanus* (Isoptera: Rhinotermitidae) Primary Reproductives by Three Chitin Synthesis Inhibitors

M. GUADALUPE ROJAS AND JUAN A. MORALES-RAMOS

USDA–ARS, Southern Regional Research Center, Formosan Subterranean Termite Research Unit, New Orleans, LA 70124

J. Econ. Entomol. 97(6): 2015–2020 (2004)

ABSTRACT Effects of the chitin synthesis inhibitors (CSIs) diflubenzuron, hexaflumuron, and lufenuron on the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, primary reproductives were studied in the laboratory. Incipient colonies were established by collecting and pairing *C. formosanus* alates and placing them in dishes containing an artificial diet. Three groups of 56 colonies each were fed with a diet containing 10 ppm of one of each of the CSIs and were compared with a control group fed with an untreated diet. All eggs oviposited by treated young queens failed to hatch at the end of 6 mo. Estimated queen fecundity was significantly lower in the lufenuron treatment compared with the control group. Fecundity of hexaflumuron-treated queens did not differ significantly from that of queens from the control group and the other treatments. Adult mortality was significantly higher in the diflubenzuron and lufenuron treatments than in the control group at the end of 6 mo., but not in the hexaflumuron treatment. All the pairs died within 8 mo. in the diflubenzuron and lufenuron treatments, even after treatment was suspended at the end of 6 mo. Mortality in the hexaflumuron treatment was significantly higher than in the control group by the end of 9 mo. The three CSIs tested eliminated reproduction in *C. formosanus* by preventing egg hatching and induced adult mortality. Possible mechanisms by which CSIs induce termite adult death are discussed.

KEY WORDS subterranean termites, incipient colonies, benzoylphenyl ureas, fecundity, growth regulators

FORMOSAN SUBTERRANEAN TERMITE, *Coptotermes formosanus* Shiraki, a native of China (Kistner 1985), was introduced into the United States after the end of World War II (Beal 1987). Since then, this termite has become the most destructive household pest in the southern United States, causing millions of dollars in damage annually (Su and Tamashiro 1987; Su and Scheffrahn 1990, 1998). The Formosan subterranean termite not only damages wooden structures but also has been reported to feed on living trees, making them structurally weak (LaFage 1987, Rojas et al. 2001).

Baits using chitin synthesis inhibitors as an active ingredient are effective at controlling subterranean termites (Su 1991, Su and Scheffrahn 1993, Rojas and Morales-Ramos 2001). Chitin synthesis inhibitors share a similar chemical structure as other benzoylphenyl ureas. Their mode of action is by interfering with the polymerization pathway of chitin, inducing accumulation of the monomer uridine diphospho-*N*-acetylglucosamine and blocking its synthesis (Beeman 1982). These benzoylphenyl ureas are slow-acting poisons (showing effects within weeks), allowing foraging termite workers to transfer the active ingredient among other members of the colony (Su and Scheffrahn 1996a).

Studies in other insects have shown that chitin synthesis inhibitors can affect the production of the peritrophic matrix of the locust *Locusta migratoria* (L.) (Clarke et al. 1977) and the blow fly *Calliphora erythrocephala* Meigen (Becker 1978). These chemicals also affect hardening of the cuticle of the boll weevil, *Anthonomus grandis grandis* Boheman (Haynes and Smith 1994) and development of the pupal integument of the yellow mealworm, *Tenebrio molitor* L. (Soltani 1987).

Reported effects of chitin synthesis inhibitors on subterranean termites mainly include defective molting of immature individuals (Su and Scheffrahn 1993, 1996b). Effects of chitin synthesis inhibitors on fecundity and survival of *C. formosanus* primary reproductives (adults) have not been reported. Chitin synthesis inhibitors affect progeny survival and egg viability in other insects, including the German cockroach, *Blattella germanica* (L.) (Koehler and Patterson 1989); *A. grandis grandis* (Wolfenbarger and Nemec 1991); the lesser grain borer, *Rhyzopertha dominica* (F.)

(Elek 1998a); and the cat flea, *Ctenocephalides felis* (Bouché) (Meola et al. 1999).

We hypothesized that chitin synthesis inhibitors may affect fecundity and egg viability of *C. formosanus* in similar ways as reported in other insect species. The objective of this study was to determine the effects of three selected chitin synthesis inhibitors on fecundity and survival of *C. formosanus* primary reproductives by using incipient colonies grown on a nutritionally balanced artificial diet.

Materials and Methods

Preparation of Experimental Units. Experimental units consisted of incipient colonies of *C. formosanus* started in the laboratory from alates captured in light traps. Incipient colonies were chosen as experimental units because measuring fecundity of *C. formosanus* queens is considerably easier in early stages of colony foundation because eggs and larvae can be counted with minimal disruption to the colonies. Also, incipient colonies allow tests with a large number of repetitions by using the limited space of environmental chambers for adequate control of experimental conditions and statistical analysis.

Alate Collection. Alate stages of *C. formosanus* were collected using an UV light trap (BioQuip 2851L, Gardena, CA). The trap was located at the northwestern end of the Southern Regional Research Center campus bordering New Orleans City Park. The trap was equipped with a timer set to turn on at 2000 hours and off at 2300 hours, covering the period of flight activity of *C. formosanus* (Henderson and Delaplane 1994) and minimizing capture of nontarget insects. Three wet paper towels were place inside the trap main container to aid the survival of collected alates. Collections were made daily starting on 10 May 2000 and ending on 31 July 2000.

Alate Pairing. Collected alates were placed inside 11.3-liter storage boxes (Rubbermaid Inc., Wooster, OH) lined with a wet paper towel until adults lost their wings, becoming dealates. Formosan subterranean termite dealates were transferred to another similar box lined with a wet paper towel. Almost immediately, male dealates began to closely follow females, forming easily identifiable pairs. Pairs of dealates were collected using an aspirator connected to a regulated laboratory vacuum source. Approximately 1000 pairs of dealates were placed individually in 12.4-ml snap-cap plastic vials (Fisher 03-338-3C, Fisher, Pittsburgh, PA).

Each termite pair was provided with a piece of the wet paper towel (Skilcraft, Industrial paper towel, National Industries for the Blind, Alexandria, VA) as a temporary source of food and water. Sections (4 by 4 cm) of white laboratory paper towels were placed inside a 500-ml beaker containing deionized water until fully saturated. The paper was compressed manually to eliminate excess water, and one of these sections was placed in each vial containing termite pairs.

Vials with termite pairs were kept at 27 ± 1°C, 95 ± 5% RH, and total darkness for 1 wk. Pairs were checked daily for mortality and the presence of eggs. Only those pairs that had produced eggs were selected for the experiments. This procedure eliminated the selection of any single-sex pairs. Pairs that failed to produce eggs were assumed to be of the same sex and were transferred back to the pairing boxes for repairing and selection.

Colony Preparation. Incipient colonies were prepared by aseptically transferring the selected termite pairs into tight-fit lid dishes (9 by 50-mm diameter) (Falcon 1006, Fisher) with 3 g of a formulated diet (Rojas and Morales-Ramos 2001). The eggs were discarded to allow all termite queens to start ovipositing simultaneously and to ensure that all eggs were produced after queens have fed on the treatments. Queens showed no difficulty producing a new set of eggs within 15 d of transfer. Pairs collected at each date were divided evenly among treatments and control to eliminate collection date bias in the analysis.

First-Stage Experiment. In total, four groups of 56 incipient colonies of *C. formosanus* were created. Three of the groups were fed with a nutritionally formulated matrix containing 10 ppm of one of three chitin synthesis inhibitors. The chemicals selected for the study were diflubenzuron (ENSYSTEX, Fayetteville, NC), hexaflumuron (Chem Service, West Chester, PA), and lufenuron (Syngenta, Wilmington, DE). The dose was chosen in an effort to simulate the amount of active ingredient transferred by foraging workers to queen, king, and larvae. However, in absence of data regarding foraging workers reaching reproductives the 10 ppm dose was chosen arbitrarily. The forth group was provided with matrix without chitin synthesis inhibitors and was designated as the control. The dishes with the incipient colonies were placed into plastic boxes (42 by 29 by 15 cm) (Sterilite Corporation, Townsend, MA) and kept at 27 ± 1°C, 95 ± 5% RH, and total darkness for the duration of the study.

Incipient colonies were monitored every 15 d for 90 d by using a stereomicroscope. Progeny were counted every monitoring period during the first 90 d of the study. The plastic dishes were thin enough to expose the nuptial chamber from either side. When the nuptial chamber was not visible, the lid of the dish was carefully twisted against the bottom just enough to expose the chamber. Mortality of pairs during the first 90 d was recorded every monitoring day. During the next 90 d, pairs were monitored monthly for colony mortality, and no progeny counts were taken until the end of the second 90-d period. In a previous study (Morales-Ramos and Rojas 2003), the high number of active workers present in the colonies after 90 d made it difficult to make accurate counts of progeny by visual examination of closed dishes. At the end of the second 90-d period (180-d total) dishes containing the incipient colonies were opened to count progeny. Total number of eggs, larvae, workers, and soldiers were recorded for each incipient colony.

Second-Stage Experiment. At the end of the first stage, incipient colonies (queen, king, and progeny) were transferred to new dishes containing 3 g of diet

as described above. During this stage, all colonies were presented with untreated diet to observe colony recovery. The colonies were placed in the environmental chamber at the same conditions above described for an additional 180-d period.

Data Analysis. Mean number of eggs, larvae, and workers + soldiers were compared between treatments by analysis of variance and Tukey–Kramer honestly significant difference test for multiple mean comparison by using JMP software (SAS Institute 2002). Colony growth was measured as the number of workers plus soldiers present at any given colony age. Changes in the number of eggs and larvae were considered less important as measures of colony growth because these stages are frequently cannibalized (Morales-Ramos and Rojas 2003).

Survival of incipient colonies (queen, king, and progeny) was measured by calculating the proportion of colonies with surviving queen and king at a given age. Colonies without queen or king were considered to be doomed. The proportion of surviving colonies was called the survival rate and had values from 0 to 1. Survival rates of incipient colonies among the different treatments were compared using the Z-test for categorical data (Ott 1984). Colony survival was compared at 60, 90, 180, and 360 d of colony age.

Queen fecundity was compared by estimating total egg production during the first 100 d of colony development. Because egg development of *C. formosanus* takes longer than 30 d at 27 ± 1°C (Morales-Ramos and Rojas 2003), adding bimonthly egg counts overestimate total egg production. Therefore, total number of eggs oviposited during the 100-d period was estimated by using the Kiritani and Nakasuji (1967) graphic integration method modified by Manly (1976). This method estimates number of individuals entering a particular stage using egg-count data taken at 15-d intervals. Total number of a given developmental stage was calculated by integrating observed densities of individuals of that developmental stage at time intervals of constant length measured in developmental rates (Manly 1976). The formula is as follows:

$$AH = \tfrac{1}{2} \sum_{i=1}^{n} (h_i + h_{i+1}) ED_i$$

$$\text{for } h_i = DR_i - DR_{i-1}$$

where AH is the total number of eggs per queen, ED_i is the density of eggs at date i, DR is the accumulated egg developmental rates at date i, and n is the last sample date. Because *C. formosanus* colonies were kept at constant 27 ± 1°C, developmental rates were calculated as 1/developmental time at 27 ± 1°C. Developmental time of *C. formosanus* eggs at 27 ± 1°C requires 35 d (Morales-Ramos and Rojas 2003).

Survival of progeny (i.e., egg viability + larvae survival) was calculated as the proportion of eggs successfully completing development from egg to worker or presoldier (fourth instar). The number of workers plus soldiers observed at 180 d of colony age was divided by the estimated number of eggs oviposited by the end of 100 d of age. Survival of progeny was compared by Z-test as was colony survival. Data are expressed as means ± SEM.

Table 1. Mean comparison of total eggs oviposited per queen during the first 100 d estimated by the graphic integration method of Kiritani and Nakasuji, and percentage progeny survival in incipient *C. formosanus* colonies treated with three chitin synthesis inhibitors at the end of 180 d of age

Treatment[a]	*n*	No. eggs[b]	No. workers + soldiers	Progeny survival[c]
Control	22	45.5 ± 4.1a	19.1 ± 3.6	37.8 ± 6.1
Hexaflumuron	17	34.8 ± 5.4ab	0.0	0.0
Diflubenzuron	13	31.2 ± 5.0ab	0.0	0.0
Lufenuron	9	23.3 ± 6.3b	0.0	0.0

Data are mean ± SEM.

[a] Chitin synthesis inhibitors at 10 ppm in an α-cellulose-based diet. The control had no active ingredient.

[b] Means with the same letter are not significantly different after ANOVA Tukey–Kramer HSD test.

[c] From egg to worker or presoldier (fourth instar). Equivalent to egg viability + larvae survival.

Results

Queens from the untreated control group oviposited significantly more eggs during the first 100 d of the experiment than queens from the lufenuron treatment ($F = 3.38$; df = 3, 57; $P = 0.024$). Mean oviposition in the hexaflumuron treatment was not significantly different than the control or the other two treatments during the first 100 d (Table 1).

Survival of progeny to a colony age of 180 d was 37.8% in the control group. These colonies had a mean of 19.1 ± 3.6 workers + soldiers at the end of the first 180 d. No progeny survived in any of the chitin synthesis inhibitor treatments (Table 1). Eggs did not hatch in any of the three treatments, and no immature stages or workers were observed in these groups at any time during the first 180 d of the study (Fig. 1).

Queens and kings exposed to diflubenzuron and lufenuron reached 100% mortality by the eighth month, and only one of the 56 pairs exposed to hexaflumuron survived to the end of the 12th mo (Table 2). Pairs exposed to hexaflumuron had a 5.5% survival at the end of ninth month. Survival in the hexaflumuron treatment was significantly greater than in the diflubenzuron and lufenuron treatments (0.0%) ($Z = 2.48$, df = 219, $P = 0.0066$), but it was significantly less than in the control group (29.1%) ($Z = 4.64$, df = 219, $P < 0.0001$) (Table 2).

At the end of 12 mo, control colonies had a mean of 24.18 ± 1.38 workers, 4.21 ± 0.27 soldiers, and 2.51 ± 0.29 larvae. There were no surviving colonies of the diflubenzuron and lufenuron treatments by the end of this period, and only one colony survived from the hexaflumuron treatment with a single worker and no other progeny at the end of 12 mo.

Discussion

The most significant effect of the three tested chitin synthesis inhibitors on *C. formosanus* biology was on

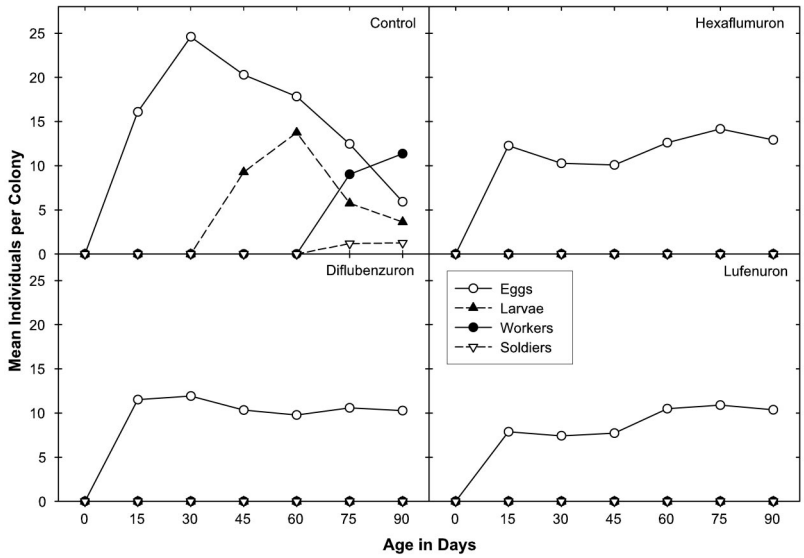

Fig. 1. Distribution of eggs, larvae, workers, and soldiers in incipient *C. formosanus* colonies fed with a nutritionally supplemented matrix and treated with three chitin synthesis inhibitors.

egg viability. Not a single larva hatched in any of the treatments during the first stage of the experiment. Microscopic examination revealed that some of the eggs developed successfully, but the first instars failed to eclose. Reduction of egg hatch has been reported in other insect species after exposure to different chitin synthesis inhibitors. Egg development in *B. germanica* was prevented when fed on baits containing 0.25% diflubenzuron (Koehler and Patterson 1989). Egg hatch was reduced by 90% in *A. grandis* after females consumed a dose of 0.000007 μg per individual of diflubenzuron or 0.009 μg per individual of penfluron (Wolfenbarger and Nemec 1991). Elek (1998a, b) observed egg mortality and reduction of egg hatching in *R. dominica* and the rice weevil, *Sitophilus oryzae* (L.), after ingestion of wheat treated with 250–750 ppm of chlorfluazuron. Egg hatch of *C. felis* declined to 64 and 2% after adult fleas fed on cattle blood treated with 0.125 and 1 ppm of lufenuron, respectively (Meola et al. 1999).

With the exception of lufenuron the chitin synthesis inhibitors tested in this study affected egg viability in *C. formosanus* at a much lower dose (10 ppm) than that reported in other insects. Previous reports on the use of chitin synthesis inhibitors on subterranean termites have been focus on the disruption of molting (Su and Scheffrahn 1993). Lenz et al. (1996) reported death of reproductives and nonhatching of eggs on *Coptotermes acinaciformis* (Froggatt). Our research is the first detailed and statistically valid report on the effects of these chemicals on Formosan subterranean termite reproduction. These results are encouraging because eliminating egg viability in a Formosan subterranean termite colony increases the chances of successful control. Estimations of oviposition rates of physogastric *C. formosanus* queens are >10,000 eggs per year (Edwards and Mill 1986). Bess (1970) reported an estimated 10,000 eggs present in a 4 yr-old *C. formosanus* nest with a physogastric queen. Higa (1981) reported a mean of 4,000 eggs in 4 yr-old colonies of *C. formosanus.* Pearce (1997) estimated an oviposition rate of ≈100 eggs per day for queens of *Coptotermes* sp. Although there is no evidence that the dose tested herein could actually reach primary queens and kings in a treated field colony, the possibility of eliminating or reducing this rate of reproduction increases the feasibility of colony elimination by the use of chitin synthesis inhibitors against *C. formosanus.*

Table 2. Comparison of incipient colony survival rates of *C. formosanus* treated with three chitin synthesis inhibitors

Treatment[a]	Colony age (mo)				
	1	3	6	9	12
Control	0.591a	0.500a	0.436a	0.291a	0.200a
Hexaflumuron	0.555a	0.446ab	0.309ab	0.055b	0.018b
Diflubenzuron	0.464ab	0.364bc	0.236bc	0.0c	0.0b
Lufenuron	0.364b	0.327c	0.164c	0.0c	0.0b

The treatment was suspended after the end of 180 d to observe colony recovery. Survival rates with the same letter are not significantly different after *Z*-test at $\alpha = 0.05$, $n = 56$.

[a] Chitin synthesis inhibitors at 10 ppm in an α-cellulose based diet. The control had no active ingredient.

Estimated number of eggs oviposited during the first 100 d was significantly lower in the lufenuron treatment group compared with the control group. These results seem to indicate that the fecundity of *C. formosanus* queens was significantly reduced during the first 100 d in this treatment. However, these results should be interpreted cautiously because the graphic method used to estimate the number of eggs oviposited assumes that eggs develop normally. Queen and king may be able to differentiate between healthy and

unhealthy eggs and cannibalize the later. The total number of eggs per colony could have been underestimated or overestimated, depending on whether the egg cannibalism was higher or unchanged in the treatment groups compared with the control. No reduction on oviposition rate has been reported after exposure to chitin synthesis inhibitors on other insects. Elek (1998b) reports significant reduction on fecundity on *R. dominica* and *S. oryzae*, but he defined fecundity as a function of progeny survival. Oviposition rate was not significantly affected in these two coleopterans by exposure to chlorfluazuron (Elek 1998b). Egg production was not affected in *C. felis* adults treated with 4 ppm of lufenuron (Dean et al. 1999).

The three chitin synthesis inhibitors tested resulted in a significantly higher mortality among *C. formosanus* primary reproductives. Diflubenzuron and lufenuron induced 100% mortality by the end of 9 mo, even when the treatment was suspended after 6 mo. Mortality in the hexaflumuron treatment was not significantly different from the control group at the end of 6 mo of treatment, but it was significantly higher at the end of 9 mo, or 3 mo after treatment termination. This indicates that *C. formosanus* dealates were unable to recover (or fully detoxify) from exposure to 10 ppm of the three chitin synthesis inhibitors tested.

These results were unexpected based on previous reports of chitin synthesis inhibitor effects on adult stages of other insects. Wolfenbarger and Nemec (1991) reported no toxic effects of diflubenzuron and penfluron on adult *A. grandis* females. Diflubenzuron did not produce significant mortality in adult *B. germanica* even at doses of 0.25% (Koehler and Patterson 1989). Elek (1998a) reported no significant increase in adult mortality of *R. dominica* and *S. oryzae* after 4-wk exposure to 750 ppm of chlorfluazuron. However, Solani 1984 reported a significant reduction in longevity of adult *T. molitor* after ingesting 500-1000 ppm of diflubenzuron, and Dean et al. (1999) reported up to 24% mortality of *C. felis* adults treated with 4 ppm of lufenuron.

One difference between this study and previous publications is the length of exposure of the adult individuals to the active ingredients. Previous studies of chitin synthesis inhibitors report exposures of ≤1 mo. Our results show that mortality of queens and kings in the diflubenzuron and hexaflumuron treatments was not significantly different from that of the control group at the end of the first month of exposure (Table 2). Only the lufenuron treatment shows significantly increased mortality rates within a month of exposure to the active ingredient. Because of their extended adult longevity, termites can be exposed to these chemicals for a substantially longer period than most other insects. Length of exposure may be a requisite for chitin synthesis inhibitors to inflict sufficient damage on adult insects to cause death. Damage to some internal organs that contain chitin may be responsible for death of adult stages. Diflubenzuron affects the synthesis of peritrophic matrix in *L. migratoria* (Clarke et al. 1977), *C. erythrocephala* (Becker 1978), and *T. molitor* (Soltani 1984). Lufenuron produces malformations of the midgut epithelium of the adult flea *C. felis* (Dean et al. 1999). More research on the effects of chitin synthesis inhibitor on internal organs of adult termites is required to fully explain the present results.

Our results show that lufenuron seems to be the most potent of the chitin synthesis inhibitors tested against primary queens and kings of *C. formosanus.* Adult mortality was significantly greater than the control in this treatment group earlier than in the other two treatments (Table 2). Hexaflumuron was the least potent of the three, showing no significant difference in adult mortality between this treatment the control group after 6 mo of exposure. However, differences in potency between hexaflumuron and diflubenzuron were slight and may not be biologically significant. Nevertheless, all three of the treatments were equally effective eliminating reproduction of incipient colonies of *C. formosanus.* Differences in potency do not mean differences in efficacy among the chemicals. However, potency is relevant, deciding the optimal dose of each chemical in the formulation of baits against subterranean termites.

Acknowledgments

We thank G. Elzen (Subtropical Agricultural Research Center, USDA–ARS, Weslaco, TX), C. Rigel (Dow Agrosciences, Baton Rouge, LA), H. Solhjoo (Southern Regional Research Center, USDA–ARS, New Orleans, LA), and N.-Y. Su (University of Florida, Fort Lauderdale, FL) for reviewing this manuscript, and S. Martin (Southern Regional Research Center) for help in literature search and retrieval of references.

References Cited

Beal, R. H. 1987. Introduction of *Coptotermes formosanus* Shiraki to the continental United States, pp. 48–53. *In* M. Tamashiro and N.-Y. Su (eds.), Biology and control of the Formosan subterranean termite. Research and Extension Series 083, College of Tropical Agriculture and Human Resources, University of Hawaii, Honolulu, HI

Becker, B. 1978. Effects of 20-hydroxy-ecdysone, juvenile hormone, dimilin, and captan on *in vitro* synthesis of peritrophic membranes in *Calliphora erythrocephala.* J. Insect Physiol. 24: 699–705.

Beeman, R. W. 1982. Recent advances in mode of action of insecticides. Annu. Rev. Entomol. 27: 253–281.

Bess, H. A. 1970. Termites of Hawaii and the oceanic islands, pp. 449–476. *In* K. Krishna and F. M. Meesner (eds.), Biology of termites, vol. II. Academic, New York.

Clarke, L., G.H.R. Temple, and J.F.V. Vincent. 1977. The effects of a chitin inhibitor - dimilin - on the production of peritrophic membrane in the locust, *Locusta migratoria.* J. Insect Physiol. 23: 241–246.

Dean, S. R., R. W. Meola, S. M. Meola, H. Sittertz-Bhatkar, and R. Schenker. 1999. Mode of action of lufenuron in adult *Ctenocephalides felis* (Siphonaptera: Pulicidae). J. Med. Entomol. 36: 486–492.

Edwards, R., and A. E. Mill. 1986. Termites in buildings their biology and control. Rentokil Limited, East Grinstead, United Kingdom.

Elek, J. A. 1998a. Treatment of adult Coleoptera with a chitin synthesis inhibitor affects mortality and development time of their progeny. Entomol. Exp. Appl. 88: 31–39.

Elek, J. A. 1998b. Interaction of treatment of both adult and immature Coleoptera with a chitin synthesis inhibitor effects mortality and development time of their progeny. Entomol. Exp. Appl. 89: 125–136.

Henderson, G., and K. S. Delaplane. 1994. Formosan subterranean termite swarming behavior and alate sex-ratio (Isoptera: Rhinotermitidae). Insectes Sociaux 41: 19–28.

Higa, S. Y. 1981. Flight, colony foundation, and development of the gonads of the primary reproductives of the Formosan subterranean termite, *Coptotermes formosanus* Shiraki. Ph.D. dissertation, University of Hawaii, Honolulu, HI.

Haynes, J. W., and J. W. Smith. 1994. Diflubenzuron plus cottonseed oil: effects on boll weevil (Coleoptera: Curculionidae) cuticle hardness, mating, and flight. J. Econ. Entomol. 87: 339–344.

Kiritani, K., and F. Nakasuji. 1967. Estimation of the stage-specific survival rate in the insect population with overlapping stages. Res. Popul. Ecol. 9: 143–152.

Kistner, D. H. 1985. A new genus and species of termitiophilous Alocharinae from mainland China associated with *Coptotermes formosanus* and its zoogeographic significance (Coleoptera: Staphylinidae). Sociobiology 10: 93–104.

Koehler, P. G., and R. S. Patterson. 1989. Effects of chitin synthesis inhibitors on German cockroach (Orthoptera: Blattellidae) mortality and reproduction. J. Econ. Entomol. 82: 143–148.

Lenz, M. P., V. Gleeson, L. R. Miller, and H. M. Abbey. 1996. How predictive are laboratory experiments for assessing the effects of chitin synthesis inhibitors (CSI) on field colonies of termites? A comparison of laboratory and field data from Australia mound-building species of termite. The International Research Group on Wood Preservation, Document No. IRG/WP 96-10143.

LaFage, J. P. 1987. Practical considerations of the Formosan subterranean termite in Louisiana: a 30-year-old problem, pp. 37–42. *In* M. Tamashiro and N.-Y. Su (eds.), Biology and control of the Formosan subterranean termite. Research and Extension Series 083, College of Tropical Agriculture and Human Resources, University of Hawaii, Honolulu, HI.

Manly, B.F.J. 1976. Extension to Kiritani and Nakasuji's method for analyzing insect stage-frequency data. Res. Popul. Ecol. 17: 191–199.

Meola, R. W., S. R. Dean, S. M. Meola, H. Sittertz-Bhatkar, and R. Schenker. 1999. Effect of lufenuron on chorionic and cuticular structure of unhatched larval *Ctenocephalides felis* (Siphonaptera: Pulicidae). J. Med. Entomol. 36: 92–100.

Morales-Ramos, J. A., and M. G. Rojas. 2003. Nutritional ecology of the Formosan subterranean termite (Isoptera: Rhinotermitidae): growth and survival of incipient colonies feeding on preferred wood species. J. Econ. Entomol. 96: 106–116.

Ott, L. 1984. An introduction to statistical methods and data analysis, 2nd ed. Duxbury Press, Boston, MA.

Pearce, M. J. 1997. Termites biology and pest management. CAB International, Willingford, Oxon, United Kingdom.

Rojas, M. G., and J. A. Morales-Ramos. 2001. Bait matrix for delivery of chitin synthesis inhibitors to the Formosan subterranean termite (Isoptera: Rhinotermitidae). J. Econ. Entomol. 94: 506–510.

Rojas, M. G., J. A. Morales-Ramos, M. A. Klich, and M. Wright. 2001. Three fungal species isolated from *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae) body, carton material, and infested wood. Fla. Entomol. 84: 156–158.

SAS Institute. 2002. JMP statistics and graphics guide. SAS Institute, Cary, NC.

Soltani, N. 1984. Effects of ingested diflubenzuron on the longevity and the peritrophic membrane of adults mealworms (*Tenebrio molitor* L.). Pestic. Sci. 15: 221–225.

Soltani, N. 1987. Diflubenzuron-induced alterations during in vitro development of *Tenebrio molitor* pupal integument. Arch. Insect Biochem. Physiol. 5: 201–209.

Su, N.-Y. 1991. Evaluation of bait-toxicants for suppression of subterranean termite populations. Sociobiology 19: 211–220.

Su, N.-Y., and R. H. Scheffrahn. 1990. Economically important termites in the United States and their control. Sociobiology 17: 77–94.

Su, N.-Y., and R. H. Scheffrahn. 1993. Laboratory evaluation of two chitin synthesis inhibitors, hexaflumuron and diflubenzuron, as bait toxicants against Formosan and Eastern subterranean termites (Isoptera: Rhinotermitidae). J. Econ. Entomol. 86: 1453–1457.

Su, N.-Y., and R. H. Scheffrahn. 1996a. A review of the evaluation criteria for bait-toxicant efficacy against colonies of subterranean termites (Isoptera). Sociobiology 28: 521–530.

Su, N.-Y., and R. H. Scheffrahn. 1996b. Comparative effects of two chitin synthesis inhibitors, hexaflumuron and lufenuron, in a bait matrix against subterranean termites (Isoptera: Rhinotermitidae). J. Econ. Entomol. 89: 1156–1160.

Su, N.-Y., and R. H. Scheffrahn. 1998. A review of subterranean termite control practices and prospects for integrated pest management programmes. Integrated Pest Manage. Rev. 3: 1–13.

Su, N.-Y., and M. Tamashiro. 1987. An overview of the Formosan subterranean termite (Isoptera: Rhinotermitidae) in the world, pp. 3–15. *In* M. Tamashiro and N.-Y. Su (eds.), Biology and control of the Formosan subterranean termite. Research and Extension Series 083, College of Tropical Agriculture and Human Resources, University of Hawaii, Honolulu, HI.

Wolfenbarger, D. A., and S. J. Nemec. 1991. Usefulness of two diphenzyl benzoyl urea insect growth regulators against the boll weevil (Coleoptera: Curculionidae). J. Entomol. Sci. 26: 466–473.

Received 10 February 2003; accepted 9 July 2004.